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THE INTRACUTANEOUS 'TYPHOIDIN' REACTION *

II. THE NATURE AND SPECIFICITY OF THE REACTION

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In the previous communication we made observations on positive skin reactions in typhoid-immune rabbits by using, as controls, glycerin broth extracts of bacteria other than typhoid. In the course of these studies we obtained results which suggested further experiments which might throw some additional light on the nature, and possibly also on the mechanism, of cutaneous hypersensitiveness and allergic skin reactions in general.

Several theories have been advanced to explain cutaneous hypersensitiveness to tuberculin, and, without additional experimental evidence, workers have used the results of studies made on other skin reactions to explain the phenomena which pertain to the skin tests which we have under consideration.

It has always been a debatable question whether the allergic tuberculin reactions are analogous to the anaphylactic reaction with serum, and whether similar skin reactions are also of the same nature. Doerr,[1] in his summary on allergy and anaphylaxis, considers the question an open one as to whether or not all the local reactions to extracts or antigenic preparations should be considered as a local anaphylaxis of the reacting individual or animal, to the protein of the disease-producing agent.

From the facts presented by numerous workers, it is certainly not unlikely that hypersensitiveness in the form of the local tuberculin reactions to various forms of antigen derived from the tubercle bacillus is, in reality, an anaphylactic phenomenon. Observations of von Pirquet[2] and others on local acquired hypersensitiveness indicate that this is more analogous to the phenomenon of sensitization than to that of hypersusceptibility to toxin. Furthermore, to support this theory a few successful attempts have been made at a passive transfer of hyper-

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[1] Handb. d. pathogen. Mikroorganismen, 1913, 2, p. 1102.

[2] Handb. d. Technik u. Methodik d. Immunitätsf., 1908, 1, p. 1050.

sensitiveness to tuberculin. However, the opponents of this conception of the nature of the tuberculin reaction have presented evidence that the active principle of tuberculin is not a specific bacterial protein, but a product closely allied to the true toxins, and that tuberculous guinea-pigs do not react as regularly to pure tuberculoprotein as to tuberculin.

From the literature on hand one may say that the experimental data are, in many respects, in accord with the theory that the tuberculin reaction is the result of sensitization. For all other forms of local allergic reaction, to mallein, 'sporotrichosine,' 'luetin,' and 'typhoidin,' the evidence thus far collected is incomplete and does not as yet permit of conclusions. In some instances, as for example in the luetin reaction, the mechanism of the skin efflorescence is, in the light of recent observations, open to various interpretations. The report of Sherrick[3] that persons treated with iodin will react with luetin, is, to be sure, of extreme interest in this connection.

Gay and Claypole,[4] in discussing the mechanism of local reactions, state that their experiments indicate that the typhoidin reaction is due to an interaction of antigen and antibody. They do not speak of a phenomenon of sensitization, but consider the positive typhoidin reaction to bear a distinct relation to protection against typhoid fever. Nichols,[5] however, regards the skin reaction as an anaphylactic phenomenon due to sensitization to typhoid protein, which is lasting and less specific than true immunity. Similarly definite are the conclusions of Kolmer[6] in recent studies on various skin tests. He speaks of cutaneous anaphylaxis to typhoidin, and luetin, and hypersensitiveness to diphtheria protein, without presenting evidence that all these reactions are the result of a mechanism similar to that of anaphylaxis. We feel that it is not quite appropriate to regard all of his reactions as manifestations of anaphylaxis, more particularly in the case of his experiments with a "broth-culture typhoidin," in which he obtained a "high percentage of nonspecific reactions."

These experiments, however, support our suggestions that all the typhoidin tests should be repeated with pure bacterial proteins if that were possible or—as long as we are unable to isolate these—with the various fractions.

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[3] Jour. Am. Med. Assn., 1915, 65, p. 404.

[4] Arch. Int. Med., 1914, 14, p. 699.

[5] Jour. Exper. Med., 1915, 22, p. 780.

[6] Jour. Immunol., 1916, 1, pp. 409, 429, 501, and 571.

Thus far only one form of antigen derived from the typhoid bacillus has been employed for the skin test on man; namely, the complex broth-culture precipitate. It must be demonstrated by further investigations whether this form of antigen is equivalent to the typhoprotein. Since in our fundamental work on typhoidin with glycerin or plain broth-culture extracts or precipitates, our results proved to be very promising (with one exception), we considered it advisable to continue their use in our next series of experiments. Also, inasmuch as nonspecific reactions described in the 1st paper, were all obtained with extracts, it was imperative that subsequent control experiments should be conducted with an antigen made in similar way.

In our pursuit, experimentally, of these nonspecific reactions, we found evidence that the typhoidin reactions are the result of sensitization, and therefore to be explained by the theories applied to anaphylactic reactions in general.

Following the observations made in connection with Groups 1 and 3, as reported in our 1st paper, we planned several experiments in which rabbits protected against various kinds of microorganisms were to be tested with typhoidin and similar extracts. For these tests the animals of Group 1 were chosen.

EXPERIMENT-GROUP 1

Two series of tests were conducted with different typhoidin preparations.

Series 1.—Eight healthy rabbits with varying degrees of immunity against B. bipolaris-septicus, Morgan's bacillus, B. dysenteriae Flexner 12, were tested on Feb. 23, 1916. From 2 to 6 months had elapsed since these animals had received their last inoculation. Some had received, over a period of 3 months, as many as 11 injections of heat-killed or living organisms. The details of immunization and of the concentration of the immune bodies at the time of the test, are given in Table 1.

The glycerin extract, Typhoidin 3 (Olsen), contained 33.3 extract units (e.u.) per 0.001 gm., whereas Fowl-typhoidin 3, tho over 1 month old, had an antigenic value of 50 e.u. per 0.001 gm.

No determinations of antigenic value were possible in the case of the extracts of staphylococci and of B. paratyphosus B (homo). The first preparation was obtained from a 48-hour-old culture. The paratyphoid extract was used in quantities of 0.001 and 0.0002 gm.

The results of the experiment with Series 1 are presented in Table 1. Rabbits 462, 487, 489, 545, 561, 562, 567, not immunized against the typhoid bacillus, developed in 48 hours a skin reaction to typhoidin, which was in every respect typical. The areolae, which were

TABLE 1

Results of Intracutaneous Tests with Typhoidin Preparations (Series 1)

Date of Tests: Feb. 23, 1916

Rabbit	Immunization	Last Injection	Serologic Tests		Hours	Typhoidin 3 Olsen 1:100	Control-Powder 3 1:10
			Agglutination	Complement-Fixation			
						(cm.)	(cm.)
462	Hemorrhagic septicemia. 6 injections of cultures and exudates	Aug. 27, 1915		0.003	16	1.8 x 1.8, R. I.	0.3 x 0.3
					24	1.9 x 1.9, W. D.	0.2 x 0.2
					48	1.9 x 1.9, R. I.	N. P.
487	B. abortivus-equinarius 9. 8 injections	Jan. 3, 1916	1:2000	0.005	16	1.5 x 1.5, R. I.	0.4 x 0.4
					24	2.5 x 2.5, R. I.	0.2 x 0.2
					48	1.5 x 1.5, R. I.	N. P.
489	B. dysenteriae Flexner 12. 11 injections of living organisms	Dec. 16, 1915	1:100	0.05	16	2.8 x 2.8, R. I.	0.2 x 0.2
					24	2.7 x 2.7, S. I.	N. P.
					48	2.2 x 2.2, I. R.	N. P.
505	B. suipestifer Voldagsen. 11 injections	Dec. 16, 1915		0.001	16	0.2 x 0.2	N. P.
					24	0.2 x 0.2	N. P.
					48	N. P.	N. P.
545	Morgan's bacillus 1. 8 injections	Nov. 4, 1915	1:800	0.005	16	1.5 x 1.5, R. I.	0.3 x 0.3
					24	1.5 x 1.5, R. I.	0.4 x 0.4
					48	1.1 x 1.1, R. I.	N. P.
561	B. dysenteriae Hiss-Y-Russell. 6 injections	Dec. 16, 1915	1:600	0.05	16	0.5 x 0.5, R. I.	0.2 x 0.2
					24	0.8 x 0.8, R. I.	0.2 x 0.2
					48	1.8 x 1.8, R. I.	N. P.
562	Fowl typhoid D 4, 7 injections of living organisms	Dec. 16, 1915	1:200	0.01	16	1.6 x 1.6, R. I.	0.3 x 0.4
					24	1.5 x 1.5, R. I.	0.2 x 0.2
					48	1.4 x 1.4, R. I.	N. P.
567	Fowl typhoid Smith. 6 injections	Dec. 16, 1915	1:600	0.001	16	2 x 2, D. O.	0.2 x 0.2
					24	2.8 x 2.8, R. I.	0.4 x 0.4
					48	2.1 x 2.1, R. I.	N. P.
609	Control		1:20	0.05	16	0.2 x 0.2	N. P.
					24	0.2 x 0.2	N. P.
					48	N. P.	N. P.

KEY TO ALL TABLES

W. D. = well-defined areola
R. I. = deep purplish, indurated area
I. R. = indurated red area
S. I. = very slight induration
D. O. = diffuse edema

TABLE 1—*Continued*

RESULTS OF INTRACUTANEOUS TESTS WITH TYPHOIDIN PREPARATIONS (SERIES 1)
DATE OF TESTS: FEB. 23, 1916

Control-Powder 4 1:10	Staphylo-coccin 4 1:100	Paratyphoidin B (homo) 3		Fowl-Ty-phoidin 3 1:100	Remarks
		1:100	1:500		
(cm.)	(cm.)	(cm.)	(cm.)	(cm.)	
0.2 x 0.2	0.4 x 0.4	1.9 x 1.9, R. I.	1.5 x 1.5, Sl. indur.	1.7 x 1.7, R. I.	Carrier on injection of B. typhosus
0.2 x 0.2	0.2 x 0.2	2.5 x 2.5, R. I.	1.4 x 1.4, R. I.	1.8 x 1.8, R. I.	
N. P.	N. P.	2.5 x 2.5, R. I.	0.9 x 0.9	1.4 x 1.4, R. I.	
0.2 x 0.2	0.2 x 0.2	0.4 x 0.4	0.4 x 0.4	2.6 x 2.6, S. I.	
N. P.	0.2 x 0.2	0.4 x 0.4	0.4 x 0.4	2.8 x 2.8, S. I.	
N. P.	N. P.	N. P.	N. P.	1.8 x 1.8, S. I.	
0.2 x 0.2	1.2 x 1.2, D. O.	0.4 x 0.4	0.2 x 0.2	0.5 x 0.5	Died from intoxication on re-injection
N. P.	1 x 1	0.2 x 0.2	0.2 x 0.2	0.2 x 0.2	
N. P.	0.5 x 0.5, R. I.	N. P.	N. P.	N. P.	
0.4 x 0.4	N. P.	0.4 x 0.4	0.4 x 0.4	0.4 x 0.4, S. I.	
N. P.	N. P.	0.2 x 0.2	0.2 x 0.2	0.2 x 0.2, S. I.	
N. P.	N. P.	N. P.	N. P.	0.2 x 0.2	
0.4 x 0.4	1.8 x 1.8, W. D.	0.9 x 0.9	0.6 x 0.6, R. I.	1.7 x 1.7, S. I.	Re-injected March 2. Carrier
0.6 x 0.6	2.2 x 2.2, indurated	1.4 x 1.4, R. I.	1.1 x 1.1	1.6 x 1.6, R. I.	
N. P.	1.7 x 1.7, R. I.	0.5 x 0.5, R. I.	0.2 x 0.2, R. .I	0.5 x 0.5	
0.2 x 0.2	0.3 x 0.3	N. P.	N. P.	N. P.	
N. P.	0.2 x 0.2	N. P.	N. P.	N. P.	
N. P.	0.2 x 0.2	N. P.	N. P.	N. P.	
0.2 x 0.2	1 x 1, D. O.	1.5 x 1.5, R. I.	0.2x 0.2	1.8 x 1.8, R. I.	Re-injected March 3. Not a carrier
N. P.	0.6 x 0.6, red area	1.8 x 1.8, R. I.	N. P.	1 x 1, R. I.	
N. P.	0.2 x 0.2	1.2 x 1.2, R. I.	N. P.	0.6 x 0.6, R. I.	
0.3 x 0.2	0.3 x 0.3	2 x 2, D. R.	0.5 x 0.5	0.6 x 0.6	
N. P.	0.5 x 0.5	1.5 x 1.5, S. I.	0.8 x 0.8, D. R. area	1 x 1	
N. P.	0.2 x 0.2	0.8 x 0.8	0.3 x 0.3	1.6 x 1.6, W. D.	
0.2 x 0.2	0.2 x 0.3	0.4 x 0.4	N. P.	N. P.	Died from intercurrent disease following injection with B. typhosus Not a carrier
N. P.	0.3 x 0.3	0.2 x 0.2	N. P.	N. P.	
N. P.	N. P.	N. P.	N. P.	N. P.	

KEY TO ALL TABLES—*Continued*

S. O. = slight edema
D. R. I. = diffusely indurated and red
D. R. area or D. R. A. = diffuse red blush
S. spot = small spot
N. P. = Needle puncture, traumatic reaction

well-defined and indurated, persisted over 48 hours. This very active preparation possessed little primary toxicity, as is shown by the reaction on Control 609 (compare also Rabbit 627, Paper III, Experiment-group 1). Rabbits 562 and 567, being immunized against fowl-typhoid organisms, and 487, against a paratyphoid-like organism, may be considered as sensitized to the bacterioprotein of organisms closely related to the typhoid bacillus, and from the observations in previous experiments, it was expected that they might respond to typhoidin in a way comparable with group reactions in serologic tests. That Rabbit 462, immunized* against a microorganism not in the least immunologically related to the typhoid bacillus should respond to typhoidin, is at first difficult to understand. The same can be said with regard to Rabbits 489, 545, and 561, which had been immunized with paradysentery or allied organisms. These reactions are, in our opinion, the expression of a hypersensitiveness of the rabbit skin to bacterial proteins or toxins, induced by extensive immunization with living organisms. This condition is nonspecific, and not explained by so-called group reactions, because Rabbits 489 and 545 reacted also to staphylococcus extracts, and Rabbits 462 and 545 to paratyphoid-B extracts. The striking gradation in the degree of reaction in these two animals to paratyphoid-B extracts, shows again how valuable quantitative tests may be in deciding whether a reaction is accidental or actually due to the powder injected.

It is quite evident that animals thoroughly immunized with bacteria not the least related immunologically, will respond to various intracutaneous glycerin extracts. The degree of the reaction depends—as already shown in Experiment-group 3 of our 1st paper—on the type of the preparations. Extracts of 48-hour-old cultures are less active and, as a rule, they give irregular results. The fowl-typhoidin preparation, being the extract of a 5-day-old culture, gave reactions in 5 of 8 animals, which reacted also to typhoidin. The reactions were less intensive, however, because the preparation was not made in shallow layers as was the typhoidin. Only in Rabbit 567, immunized against fowl-typhoid organisms, did the reaction persist for 48 hours; in the other control animal, 562, the areola disappeared after the first 24 hours.

Three animals (489, 545, and 562) reacted with a weak staphylococcus extract; in 545 the reaction was in accord with the general hypersensitiveness to other bacterial proteins. In Rabbits 489 and 562 the reactions were so slight and transitory as to be doubtful, and possibly were due to faulty technic or traumatism.

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* The serum of this animal protected mice and rabbits against an infection with large doses of peritoneal exudate containing B. bipolaris-septicus.

Series 2.—Nine healthy rabbits, possessing a fair degree of immunity against B. typhosus (320, 322), fowl-typhoid organisms (404, 426), B. pullorum (410), B. abortus-equi (479), yeasts (424, 510), and organ extracts (560), respectively, were tested with various glycerin extracts. From 129 to 250 days had elapsed since these rabbits had received their last injections of antigen. Most of the animals were immunized with more than 8 injections, some receiving as many as 19. The details of the condition of immunity and of the procedure of immunization are given in Table 2.

Two rabbits (597 and 604) were infected with tuberculosis and sporotrichosis, respectively; subsequent postmortem examination demonstrating that the infection had taken place. Only 2 rabbits (662 and 663) were used as controls.

The typhoidin preparations E were used in the tests. Preparation E-I consisted of a 25-day-old glycerin potato broth, extracted and precipitated as usual; this preparation had an antigenic value of 20 e.u. per 0.001 gm. of powder. One portion of the broth culture was heated at 60 C. and autolyzed at 45 C. for 4½ hours, then filtered through hardened paper, preserved with 0.5% carbolic acid, and used undiluted as Antigen E-II.

A staphylococcus extract was prepared in the same manner as E-I. The two paratyphoid A and B powders, marked P.C., had been similarly prepared, but were about 1 month old when used. They proved to be specific and fully potent when subsequently tested.

The results of this series of tests are shown in Table 2. The typhoid-immune rabbits (320 and 322) gave fair skin reactions with both typhoidin preparations; the preparation E-II gave somewhat more intense areolae, and the induration persisted in Rabbit 322 for at least 48 hours. The same rabbit also responded more intensively to the paratyphoid-A and -B extracts than did Rabbit 320. Both rabbits had been immunized by the same process, and serum immune bodies were present in about equal concentration.

The rabbits immunized with bacterial antigens other than typhoid, all gave reactions with typhoidin which could not be distinguished from the skin reactions of Rabbits 320 and 322. However, Preparation E-I produced again somewhat less intense reactions. In 3 hyperimmune rabbits (404, 410, and 424) no dermatic response to typhoidin could be recorded. Of these three rabbits, 410 reacted to the paratyphoid-B extract, 424 to the staphylococcus extract and both typhoidin preparations, while 404 reacted to neither. In some of the rabbits, for example, 510 and 479, the diameter of the areola was greater and its induration more intense than in the typhoid-immune rabbits, and yet both these animals were immunized with organisms which botani-

TABLE 2

RESULTS OF INTRACUTANEOUS TESTS WITH TYPHOIDIN PREPARATIONS (SERIES 2)
DATE OF TESTS: APRIL 25, 1916

Rabbits	Immunization or Infection	Time Interval Since Last Injection	Serologic Tests		Hours
			Agglutination	Complement-Fixation	
320	Infection with B. typhosus 3. 7 injections	Nov. 8, 1915 153 days	1:4000	0.005	24 48
322	B. typhosus 15. 7 injections	Nov. 8, 1915 153 days	1:1000 —2000	0.005	24 48
404	Bacillus of fowl-typhoid Smith. Since March, 1915, 19 injections	Sept. 22, 1915 192 days	1:80	<0.02	24 48
410	B. pullorum III, 17 injections	Sept. 22, 1915 192 days	1:80	0.02	24 48
424	Yeast I. V. 14 injections	Sept. 14, 1915 201 days	1:20 (Olsen)		24 48
426	Bacillus of fowl-typhoid -A. P. Since April, 1915, 14 injections	Sept. 22, 1915 192 days	1:100	<0.02	24 48
479	B. abortus eqi. 8 2 injections of toxin	Aug. 12, 1915 250 days	1:60	0.05	24 48
510	Yeast Curtiss. 5 large doses	Sept. 14, 1915 250 days	1:40 (Olsen)	0.01 (50%)	24 48
560	Submaxillary gland of dog	Dec. 16, 1915 129 days	1:40	0.05 (50%)	24 48
597	Tuberculosis	Feb. 17, 1916 Infected	(1:400?)	0.02 (50%)	24 48
604	Sporotrichosis	Feb. 17, 1916 Infected	1:40	0.1	24 48
662	Control		1:40	0.05 (50%)	24 48
663	Control		1:5	0.05 (50%)	24 48

TABLE 2—*Continued*

RESULTS OF INTRACUTANEOUS TESTS WITH TYPHOIDIN PREPARATIONS (SERIES 2)
DATE OF TESTS: APRIL 25, 1916

Typhoidin E		Control-Powder E 1:50	Staphylococcin E 1:100	Paratyphoidin A, P. C. 1:100	Paratyphoidin B, P. C. 1:100	Remarks
1:100	Broth Undiluted					
(cm.) 1.3 x 1.3 0.8 x 0.8	(cm.) 1.8 x 1.8 1.3 x 1.3	(cm.) 0.8 x 0.8 Red spot	(cm.) Red spot 0.6 x 0.6	(cm.) 1.3 x 1.3 Red spot	(cm.) 1.5 x 1.5 1.5 x 1.5	Re-injected on May 3. Carrier
1.8 x 1.8 1.8 x 1.8	2.3 x 1.6 1.8 x 2.4	Red spot Red spot	Red spot 0.8 x 0.8	1 x 1 0.8 x 0.8	1.8 x 1.8 1.8 x 1.8	Re-injected on May 3. Not a carrier
N. P. N. P.	1.8 x 1.8 0.8 x 0.8	N. P. N. P.	0.9 x 0.9 Red spot	N. P. N. P.	N. P. N. P.	Re-injected on May 3. Not a carrier
Red blush Red blush	1 x 1 1.3 x 1.3	N. P. N. P.	N. P. N. P.	N. P. N. P.	1.6 x 1.6 2.1 x 2.1	Re-injected on May 3. Died from infection May 12
N. P. N. P.	D. R. A. 1.8 x 1.8	N. P. N. P.	1 x 1 0.5 x 0.5	1.7 x 1.7 1.7 x 1.7	1.3 x 1.3, yellow center 0.5 x 0.5	
1.5 x 1.5 1.9 x 1.9	1.4 x 1.4 1.5 x 1.5	N. P. N. P.	0.8 x 0.8 1 x 1	0.8 x 0.8 0.8 x 0.8	Red area 1.8 x 1.8	Re-injected on May 3. Not a carrier
Red area 1.9 x 1.9, indurated white area	Indurated red area 2.1 x 2.1	N. P. N. P.	N. P. N. P.	N. P. N. P.	N. P. N. P.	
Red area 2.4 x 1.5, R. I.	Red area 2.1 x 2.1, R. I.	N. P. N. P.	N. P. N. P.	N. P. N. P.	N. P. N. P.	
N. P. N. P.	2 x 2, nodule, indurated Red spot	N. P. N. P.	N. P. N. P.	N. P. N. P.	N. P. N. P.	
1.3 x 1.3, R. I. 1.5 x 1.5, R. I.	1.5 x 1.5 1.9 x 1.9	Red area 0.6 x 0.6	N. P. 0.5 x 0.5	1.2 x 1.2 1.4 x 1.4	1 x 1 1 x 1	
1.5 x 2 1.7 x 1.7	1.8 x 1.3 1 x 1.5	N. P. N. P.	1.3 x 1.3 0.4 x 0.4	2.3 x 1.5 1.4 x 2	1.5 x 1.5 1.7 x 1.7	
1.5 x 1.5, areola puffy 0.6 x 0.6, red area	N. P. Red area	N. P. N. P.	N. P. N. P.	N. P. N. P.	N. P N. P.	
N. P. N. P.	N. P. N. P.	N. P. N. P.	N. P. N. P.	Areola 0.5 x 0.5 N. P.	N. P. N. P.	

cally are not related to B. typhosus. It is possible that these skin reactions were due to toxic substances in the broth, for one control, 662, gave a slight reaction in the first 24 hours with E-I. In the immunized rabbits, however, well-marked and well-defined reactions were present after 24 hours, at a time when, in our experience, the toxogenic effect of the typhoidin powders would, as a rule, have disappeared. One might offer another and possibly better supported explanation for these reactions in that these rabbits became generally sensitized to some bacterial or vegetable (yeast) protein common to all organisms, and that the skin phenomena were the result of an anaphylactic reaction. For most of the hyperimmune rabbits this probably holds true, but for Rabbit 479 (Strain 8) this is, at first glance, unlikely, because the amount of bacterial protein inoculated was very small, and, as we became convinced from other experiments, was not sufficient to sensitize guinea-pigs. We found later, however, that even very small amounts of typhoproteins introduced intradermally for tests in normal healthy rabbits, are capable of producing a condition which gives rise to typical skin reactions on subsequent application of typhoidin. Rabbit 479 had been inoculated subcutaneously with a toxic broth filtrate of an organism closely allied to the paratyphoid-A bacillus, and in this case a group reaction with the typhoid antigen was therefore also possible.

In Experiment-group 1, Series 2, it is not apparent how much the degree of immunity and the time interval between the last injection and the skin test influenced the intensity of the reaction. From other experiments on rabbits, it is evident, however, that cutaneous hypersensitiveness if once acquired is never entirely lost, but will show frequent fluctuations in intensity, which are probably the result of factors unknown. A certain percentage of rabbits, just as has been observed in man, will never develop a hypersensitiveness even when injected at regular intervals with large amounts of bacterial substances. It is not unlikely that Rabbit 404 exemplifies this point, but it is also possible that the refractory condition of this animal was the outcome of over-immunization, which may result, as is generally known, in complete loss of power to produce antibodies.

Rabbits 597 and 604, infected with tuberculosis and sporotrichosis, respectively, gave well-defined skin reactions. It is interesting to note that the extensively tubercular rabbit, 597, also possessed agglutinins for B. typhosus. These observations support the findings of Kraus[7] and others in regard to tubercular human beings.

Cutaneous hypersensitiveness of tubercular persons to tuberculin and other bacterial extracts has always been one of the strong features which supported the conception of the tuberculin reaction as nonspecific. We have made further inquiries into the nature of these reactions on other animals, and have collected further interesting data which will be considered later, in the discussion of these experiments. The presence of agglutinins cannot be the outcome of a mixed infection, as in man,[8] but is probably the result of a nonspecific response of the antibody-forming organs, and represents a condition of antibody-production to which our attention has been recently directed by nonspecific intravenous treatment with colloidal substances (Jobling[9]).

The animals of this group demonstrate the hitherto unknown fact that a certain percentage of rabbits, highly immunized with various living and dead bacterial suspensions, will give skin reactions following the intradermal introduction of glycerin broth-culture extracts of microorganisms which immunologically are in no way related to the bacteria used in the immunization. These nonspecific reactions persist, frequently, up to the 48th hour. They may be the result of cutaneous hypersensitiveness to certain elements of the bacterial protein. Some are due, perhaps, to individual hypersusceptibility to toxic elements of the glycerin extract. These conditions of the skin have thus far been noted in immunized and infected rabbits only.

EXPERIMENT-GROUP 2

The observations made in Group 1 have been verified in another series of tests and enhanced by the use of different types of preparations. In the light of the explanation which Stokes[10] offered for the luetin reactions, it was considered of interest to test also a colloidal substance, like agar, in a parallel series with the regular extract powders. Group 2 represents one of the experiments prepared for this purpose.

Twelve healthy rabbits previously immunized with various bacterial suspensions and vaccines, and 3 control animals (646, 647, 648) were used in the test. Rabbits 328, 329, and 300, which had been intensely vaccinated against the

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[7] München. med. Wchnschr., 1910, 57, p. 437.

[8] See the publications of Roth, Centralbl. f. Inn. Med., 1910, 21, p. 1, and Krenker, München. med. Wchnschr., 1909, 56, p. 1017.

[9] Jour. Am. Med. Assn., 1916, 66, p. 1753.

[10] Jour. Infect. Dis., 1916, 18, p. 415.

TABLE 3

RESULTS OF INTRACUTANEOUS TESTS WITH TYPHOIDIN PREPARATIONS (GROUP 2)
DATE OF TESTS: APRIL 17, 1916

Rabbit	Immunization	Date of Last Injection	Serologic Tests		Hours
			Agglutination	Complement-Fixation	
313	Dog serum (27.5 c.c.) 4 injections	Jan. 25, 1916	0	0.1 (50% with B. typhosus)	24 48
316	Dog serum (27.5 c.c. I. V.) 4 injections	Jan. 25. 1916	1:20 (Olsen)	0.1 (50% with B. typhosus)	24 48
328	B. typhosus 20 (toxic strain) 6 injections	Nov. 8, 1915	4/17 1:1000	0.02	24 48
329	B. typhosus 32 (toxic strain) 6 injections	Nov. 8, 1915	4/17 1:1000	0.02	15 24 48
330	B. typhosus 38 7 injections	Nov. 8, 1915	4/17 1:1000—2000	0.01	15 24 48
447	B. bipolaris-septicus 97 6 injections of living organisms and exudate	Sept. 28, 1915	4/17 0 (bipolar 49) 1:20 (B. typhosus)	0.01 (with Iowa I)	24 48
463	Hemorrhagic-septicemic vaccine 7 injections	Sept. 28, 1915	4/17 1:200 (Iowa I) 1:200 (B. typhosus 49)	0.1 (50% with B. typhosus) 0.003 (with B. bipolaris)	24 48
484	Yeast (sputum) 6 injections	Sept. 14, 1915	4/17 0	0.05 (50% with B. typhosus)	24 48
492	B. pullorum (Berkeley) 13 injections	Dec. 3, 1915	4/17 1:60	0.05 (B. pullorum)	24 48
495	B. avisepticus 5 injections	Sept. 14, 1915	4/17 0 (B. avisepticus) 1:40 (B. typhosus)		24 48
600	Hemorrhagic-septicemic vaccine 1 injection	Feb. 17, 1916	>1:200 (Iowa I) 0 (B. typhosus 49)	>0.003 (B. bipolaris 49)	24 48
611	B.-abortus toxin 2 injections (6 c.c.)	March 1, 1916	1:200 (B. abortus-equi)	0.02 (B. abortus-equi)	24 48 72
646	Control		0 (Olsen)	0.05 (50% with B. typhosus)	24 48
647	Control		0 (Olsen)	0.1 (50%)	24 48
648	Control		1:20 (Olsen)	0.1 (50%)	24 48 72

TABLE 3—*Continued*

RESULTS OF INTRACUTANEOUS TESTS WITH TYPHOIDIN PREPARATIONS (GROUP 2)
DATE OF TESTS: APRIL 28, 1916

Typhoidin 15		Control-Powder 15 C 1:100	Fowl-Typhoidin 4 1:100	Control-Powder 1:10	Agar (0.5%)	Abortin 1:100	Remarks
1:50	1:100						
(cm.)	(cm.)	(cm.)	(cm.)	(cm.)	(cm.)	(cm.)	
1.8 x 1.8, atypical	0.9 x 0.9	N. P.	2.2 x 2.2	0.9 x 0.9	0.8 x 0.8		
2 x 2, atypical	0.8 x 0.8		2 x 2	0.5 x 0.5	0.5 x 0.5, whitish nodule		
0.9 x 0.9, whitish	N. P.	Red spot	1.5 x 1.5, R. I.	0.5 x 0.5, yellow	N. P.		
0.5 x 0.5, nodule	N. P.	Red spot	1.4 x 1.5, R. I.	Red spot	N. P.		
2 x 2, I. R.	2 x 2, I. R.	Red spot	Red spot	1.8 x 1.8	N. P.	N. P.	Re-injected on April 25. Carrier
1.6 x 1.6, I. R.	1.5 x 1.5, I. R.	N. P.	Red spot	1.4 x 1.4	0.6 x 0.6	N. P.	
Red area	Red spot	N. P.	0.9 x 0.9	0.5 x 0.5	0.9 x 0.9		Re-injected on April 25. Died from typhoid relapse
1 x 1, red area	Red spot	N. P.	1.2 x 1.2	Red spot	0.9 x 0.9		
0.8 x 0.8	Red spot	N. P.	1.8 x 1.8	Red spot	1 x 1		
Red area	Red area	N. P.	Red spot	Red spot	Small nod.		Re-injected on April 25. Not a carrier
2.2 x 2.2	N. P.	N. P.	Red spot	N. P.	Small nod.		
1.4 x 1.4	N. P.	N. P.	N. P.	N. P.	N. P.		
1.5 x 1.5	1.4 x 1.4	Red spot	1.5, D. R. A.	0.5 x 0.5	Small nodule	N. P.	
Red spot	1.4 x 1.4	N. P.	1.6 x 1.6, D. R. A.	Red spot	Small nodule	N. P.	
N. P.	N. P.	N. P.	N. P.	N. P.	N. P.	N. P.	Clinically sick, emaciated
N. P.	N. P.	N. P.	N. P.	N. P.	N. P.	N. P.	
Red area	Red area	N. P.	1.1 x 1.1	N. P.	N. P.	N. P.	
1.2 x 1.2, whitish	0.6 x 0.6, slight reddening	N. P.	1.1 x 1.1	Red spot	N. P.	N. P.	
2 x 2	1.9 x 1.9	0.5 x 0.5	1.4 x 1.4	Red spot	0.9 x 0.9	0.9 x 0.9, red area	
1.7 x 1.7	0.8 x 0.8	Red spot	1.7 x 1.7	Red area	Red nodule	Nodule	
0.5 x 0.5, D. R. A.	0.5 x 0.5, red area	Red spot	0.2 x 0.2, nodule	Red area	N. P.	N. P.	
Nodule	Nodule	N. P.	N. P.	Small nodule	N. P.	N. P.	
D. R. A.	D. R. A.	N. P.	1.8 x 1.8	Red area	0.9 x 0.9, R. I.	N. P.	
0.1 x 0.1, red spot	0.1 x 0.1, red spot	N. P.	1.3 x 1.3	Red spot	0.6 x 0.6	N. P.	
Red spot	N. P.	N. P.	0.5 x 0.5	0.8 x 0.8	0.5 x 0.5	N. P.	
1.4 x 1.4, atypical	N. P.	N. P.	0.5 x 0.5	0.5 x 0.5	0.5 x 0.5	N. P.	
Red nodule	N. P.	N. P.	Red spot	Red spot	N. P.	N. P.	
N. P.	N. P.	N. P.	N. P.	0.3 x 0.3	N. P.	N. P.	
N. P.	N. P.	N. P.	N. P.	N. P.	N. P.	N. P.	
N. P.	N. P.	N. P.	N. P.	0.5 x 0.5	N. P.	N. P.	
N. P.	N. P.	N. P.	N. P.	N. P.	N. P.	N. P.	
Red area	Red spot	N. P.	0.4 x 0.4	0.4 x 0.4	N. P.	N. P.	
1.5 x 1.5	1.1 x 1.1	N. P.	0.4 x 0.4	Red spot	N. P.	N. P.	
N. P.	Red spot	N. P.	N. P.	N. P.	N. P.	N. P.	

typhoid bacillus, served as controls for the typhoidin preparation. One hundred and sixty-one days had elapsed since the last inoculation, and their immunity as judged from the amount of complement-fixing antibodies present in the serum, was correspondingly slight; the agglutinins, however, were still present in fair amounts.

The other rabbits had been immunized with B. bipolaris-septicus (447, 463, 495, 600), a yeast isolated from sputum (484), dog serum (313, 316), B. pullorum (492), and B. abortivius-equinarius (611). For details regarding the immunization and the time interval following the last injection, the reader is referred to Table 3.

The glycerin extract of the typhoid bacillus, Typhoidin 15, with an antigenic value of 5 e.u., was a low-grade preparation obtained by growing the strain Olsen in a glycerin veal potato broth for 24 days. The unfiltered culture, which showed a heavy sediment, was concentrated to one-tenth its volume and precipitated as usual. The powder possessed a grayish tinge, was granular, and dissolved only with difficulty.

Fowl-typhoidin 4 was a preparation made with Witte's peptone, which had been grown for 5 days at 37 C.; its value was 20 e.u. per 0.001 gm. of powder. The abortin was a glycerin extract of cultures of B. abortus from 10 to 12 weeks old, and had been prepared in the usual manner. The colloid was a 0.5% solution of agar in salt solution, sterilized at 120 C.

The results of this experiment are described in detail in Table 3. Typhoidin 15 in amount of 0.001 gm. proved to be a weak antigenic substance. Only Rabbit 328 of 3 typhoid-immune animals (328, 329, 330) developed readable reactions; 2 other rabbits (447 and 492), immune to B. pullorum and B. bipolaris-septicus, respectively, gave positive typhoidin tests however. The powder was not absolutely inert in one normal rabbit (648)—one of those animals showing an individual hypersensitiveness to the toxic properties of the broth constituents.

In the amount of 0.02 gm., the powder produced specific reactions in 2 typhoid-immune rabbits (328 and 330), but in 2 rabbits (313 and 611) not immunized against the typhoid bacillus, atypical areolae appeared which deserve further description and discussion. A small hyperemic, slightly warm area developed in the first 24 hours; induration was slight, or of such degree that it was only recognized with difficulty. Inside this area was a whitish center, which enlarged to a small nodule in the next 48 hours. Similar reactions were noted in some rabbits at the site of injection of Broth-control 4, which was used in an exceptionally high concentration. It is quite evident that most of the rabbits which reacted to the agar solution reacted also to these concentrated powder suspensions, which were rather insoluble in salt solution, turbid and milky, and had all the properties of a colloidal solution. It is possible that these atypical reactions are the result of a mechanism which Stokes has discussed and explained on the basis of his experimental work. Reasoning from Jobling's and Petersen's[11] conception of anaphylaxis, Stokes has formulated a theory that the antiferment-absorbing effect of colloids is responsible for a local disturbance of the protease balance with production of toxins which, in turn, cause the vascular disturbances characteristic of an intracutaneous reaction in man.

The rabbits which developed these nonspecific skin reactions developed good typical areolae also with the fowl typhoidin. We suspect, therefore, that these cutaneous reactions, typical as well as atypical, are the result of individual conditions, in part due perhaps to the immunization and therefore similar to those noted and described in previous experiments.

For the first time we noticed that a weak typhoidin preparation failed to produce specific skin reactions in some properly immunized animals. In Rabbit 329 not even 0.02 gm., and in 330 only this amount, called forth a typical reaction. According to the agglutination titer these rabbits had developed about the same amounts of immune substances. It is of interest to note at this time that in a subsequent immunity test, Rabbit 328, which gave a well-marked typhoidin reaction, became a carrier (gall-bladder). Rabbit 330 resisted the infection and Rabbit 329, which reacted only to fowl typhoidin, succumbed in 23 days after the intravenous injection of living typhoid bacilli, to an acute typhoid relapse.

Fowl-typhoidin 4, which according to previous experiments called forth specific skin reactions in typhoid-immune rabbits, produced in the test under consideration a typical and persistent areola in 1 rabbit only (329). A specific reaction was also noted in Rabbit 492. The nonspecific response in 313, 316, 484, and 600 is, as already pointed out, the result either of intensive sensitization to bacterial proteins, or of metabolic changes following the injection of foreign proteins (313, 316).

The positive reactions in Rabbits 313 and 316 are the most difficult ones to explain. It is not unlikely that 27.5 c.c. of dog serum produced nonspecific changes and conditions of hypersusceptibility to bacterial extracts which are perhaps similar to those produced in hyperimmunization with bacteria. The inflammatory character was well marked in the reaction to fowl typhoidin, and at first it was thought that these skin reactions were due to the primary toxicity of the powder, or to traumatism. But persistence of the reaction up to the 48th hour, and slight or negative results in the control rabbits, recorded in Tables 1 and 3, do not support this contention.

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[11] Jour. Exper. Med., 1915, 21, p. 239; 1914, 20, p. 37.

The abortin in dose of 0.001 gm. was inert in all rabbits; some indications of a reaction were seen in the hyperimmune rabbit 492, which responded, as stated, to all the bacterial proteins inoculated.

Group 1 permits certain conclusions. A 24-day-old glycerin-potato-broth culture of B. typhosus produces a typhoidin which has low antigenic value, and which accordingly is only active in dilutions of 0.02 gm. On account of its insolubility in salt solution, it may act on intracutaneous injection as other colloids like agar, giving rise to atypical reactions. Rabbits immunized with bacterial or yeast protein and apparently also those immunized with animal (dog) protein, otherwise in good health, acquire cutaneous hypersusceptibility to glycerin extracts of bacterial broth cultures. The intensity of reaction is directly influenced by the amount of protein introduced. Hyperimmune animals which have recovered from an infection (447) will react with every glycerin extract used in the experiment.

EXPERIMENT-GROUP 3

Inasmuch as it was not quite clear what caused these nonspecific reactions, which had occurred in every experiment thus far conducted, and inasmuch as our interpretation of them was based on the idea of sensitization, we conducted some experiments in which the disturbing factor of broth constituents in the typhoidin powder was eliminated. Typhoidin powders carefully redissolved, filtered, and again precipitated, constituted one antigen, and an autolysate of the typhoid bacillus grown on agar, the other. The latter represented a pure preparation of the various typhoproteins of the bacillus.

At present we shall report one experiment (3), and we hope to show in the near future that the suggestion therein recorded will be of value in the discussion of sensitization in general.

Thirteen rabbits previously injected by various methods with animal and bacterial proteins or living bacteria, were used in this experiment. Five rabbits (749 to 753), corresponding in age and size to the test rabbits, served as controls. Rabbits 691 and 692 had been immunized, by a rapid method with 5 injections, against 2 different strains of B. typhosus. Forty days after the last injection they had received another dose of living typhoid bacilli. They were tested 24 days after the last injection.

Rabbits 716, 717, and 719 had been immunized in similar manner with typhoid, and recently isolated paratyphoid-A organisms, respectively, and were tested 14 days after the last injection. One rabbit (711) had been immunized with living paradysenteriae bacilli (Strain 9). It had received 5 intravenous injections and was tested 7 days after the last injection.

About 14 to 48 days prior to the skin tests, several rabbits (703, 704, 705, 706, 718, 741) had been injected directly in the gallbladder with small doses of living dysentery, paradysentery, and typhoid bacteria. Most of these animals showed a slight response to the infection as judged by the serum immune bodies.

Two rabbits (620 and 695) had received several intravenous injections of sheep cells and had produced a low-grade hemolysin. A final injection had been given 3 days before the test. The typhoidin preparations tested in this experiment were, for reasons already stated, different from those hitherto described.

Preparation I^a.—This was a 5-day-old glycerin rabbit-broth-culture extract of an old typhoidin strain which had been passed several times through the gallbladder of a rabbit.

The concentrated extract, before precipitation, was filtered through Chardin paper. The final product contained 20 e.u. per 0.001 gm. and, according to Chart 1 in our 1st paper, it was a good antigen.

Preparation I^b.—This was the purified precipitate of I^a. The powder was 3 times redissolved in salt solution and again precipitated with absolute alcohol. A somewhat more concentrated antigen was thus obtained, the antigenic value of the powder being 33.3 e.u. per 0.001 gm.

Preparation II^a.—This was prepared with a recently isolated strain of B. typhosus, but was unfiltered. It had an antigenic value of 20 e.u. per 0.001 gm. and was a poor antigen. As controls for the rabbit-broth and peptone contents, the concentrated and precipitated rabbit broth was used.

Preparation V.—This was obtained as follows: The 48-hour-old agar surface growth of several Blake bottles of the same strain of B. typhosus as used in Preparation II^a, was suspended in distilled water, shaken for one-half hour, and heated for 1 hour at 60 C. The autolysate was precipitated with alcohol and dried in vacuo to a scaly mass. This typhoprotein was soluble with difficulty in carbolized salt solution, and represented in the dilution of 1 : 100 a thick milky suspension. In a dilution of 1 : 1000 it was turbid and, on standing, a whitish débris settled out.

Inoculated intravenously into a rabbit, it caused, in less than 24 hours, acute intoxication in dose of 0.1 gm.; small doses like 0.01 gm. provoked loss of weight (100 to 200 gm.) and temporary illness. As a whole, however, its toxic effect on the animals by intravenous injection was less compared with that of some of the glycerin extracts. In amount of 0.001 gm. subcutaneous injections of this concentrated bacterioprotein caused abscess-formation and induration. The preparation was exceedingly antigenic, containing 333 e.u. per 0.001 gm.

Tuberculin Bovine C.—This was the alcoholic precipitate of an old concentrated, but not filtered, culture of a bovine type of tubercle bacillus. To determine the effect of the tubercle protein the powder was used in the concentration of 0.02 gm.

Pork Protein.—This was a 10% salt solution of pork protein prepared from the alcoholic precipitate obtained according to the method of Wodenhouse.[12]

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[12] Boston Med. and Surg. Jour., 1916, 175, p. 195.

TABLE 4

RESULTS OF INTRACUTANEOUS TESTS WITH TYPHOIDIN (GROUP 3)

Rabbit	Immunization	Last Infection	Serologic Tests		Hours	Typhoidin IIa 1:100	Typhoidin Ia 1:100
			Agglutination	Complement-Fixation			
620	Sheep hemolysin	Oct. 7, 1915	1:20 (H-125)		24	(cm.) 0.6 x 0.6, not indurated	(cm.) 0.8 x 0.8, not indurated
					48	0.2 x 0.2, nodule	0.2 x 0.2, nodule
					120	....	....
691	B. typhosus Nelson 6 injections	Sept. 16, 1916	10/7 1:6000	(0.0005) 0.001	24	1.4 x 1.4, well-defined nodule	0.9 x 0.9, well-defined nodule
					48	1.1 x 1.1	0.9 x 0.9, nodule
					120	....	....
692	B. typhosus 323 6 injections	Sept. 16, 1916	10/7 1:6000	>0.0005 0.002	24	1.3 x 1.3, R. I. not well defined	1.4 x 1.4, R. I., W. D.
					48	0.5 x 0.5,	1.2 x 1.2,
					120	well-defined Nodule	W. D. Nodule
695	Sheep hemolysin	Oct. 7, 1916	1:20 (H-125)		24	Red spot	Red spot
					48	Red spot, 0.5 x 0.5	Red spot
					120	....	....
703	B. paradysenteriae 9 Gallbladder-injection	Sept. 2, 1916	1:100 to 1:200		24	Red spot	1 x 1, red area
					48	Red spot	Red spot
704	B. dysenteriae Shiga Do. Gallbladder-injection	Sept. 2, 1916	1:80		24	Rde spot	Red spot
					48	Red spot	Red spot
705	B. paratyphosus A, Fraser Gallbladder-injection	Sept. 2, 1916	10/7 1:2000	0.03	24	Red spot	1.4 x 1.4, W. D.
					48	Red spot	1.5 x 1.5, W. D.
					120	....	....
706	B. dysenteriae Mt. Desert Gallbladder-injection	Sept. 2, 1916	1:60		24	1.6 x 1.6, D. R.	1.1 x 1.1
					48	Red area	Red area
					120	....	....
711	B. dysenteriae Honolulu 9 intravenously		1:2000 —1:6000	0.001	24	Red spot	1 x 1, no induration, whitish patch
					48	Whitish area 0.5 x 0.5	....
					120	....	....
716	B. typhosus Mexico 6 injections	Sept. 26, 1916	1:4000	>0.0005	24	1.8 x 1.8, yellow center	1.9 x 1.9, R. I.
					48	1.1 x 1.1, nodule	1.4 x 1.4, R. I.
					120	0.5 nodule	Nodule

TABLE 4—*Continued*

RESULTS OF INTRACUTANEOUS TESTS WITH TYPHOIDIN (GROUP 3)

Typhoidin I-b 1:100	Control-Powder 1:25	Typhoidin V		Tuberculin B. C. 1:50	Pork Protein 1:10	Remarks
		1:100	1:1000			
(cm.)	(cm.)	(cm.)	(cm.)	(cm.)	(cm.)	
1 x 1, not indurated	0.2 x 0.2	2 x 2, R. I.	0.3 x 0.3, R. I.	0.8 x 0.8, R. I.	N. P	
0.2 x 0.2, nodule	N. P.	1.4 x 1.4, R. I.	0.1 x 0.1, nodule	0.3 x 0.3, nodule	N. P.	
....	....	nodule	....	nodule		
1.4 x 1.4, well-defined nodule	0.8 x 0.8	1.5 x 1.5, well-defined nodule	0.9 x 0.9, nodule	0.8 x 0.8, raised nodule	N. P.	
1.4 x 1.4, well-defined nodule	N. P.	1.4 x)1.4, well-defined nodule	0.5 x 0.5, nodule	1 x 1 nodule size of a pea	N. P.	
....	....	Nodule				
0.6 x 0.6, well-defined nodule	0.5 x 0.5, not well defined	1.8 x 1.8, very well defined	0.7 x 0.7, W. D.	0.5 x 0.5, raised induration	N. P.	
0.7 x 0.7, well-defined nodule	N. P.	1.8 x 1.8, R. I.	0.9 x 0.9, W. D.	0.7 x 0.7, well-raised nodule	N. P	
Nodule	....	Nodule	Nodule	Nodule size of a pea		
0.4 x 0.4	0.6 x 0.6	1 x 1	0.6 x 0.6	0.7 x 0.7	N. P	
1.2 x 1.2	N. P.	1.9 x 1.9, R. I.	N. P.	0.2 x 0.2	N. P.	
....	....	Nodule				
N. P.	N. P.	1.7 x 1.7	1.2 x 1.2	0.8 x 0.8	N. P	
N. P.	N. P.	1.8 x 1.8	0.8 x 0.8	0.5 x 0.5	N. P.	
1.5 x 1.5, R. I.	0.5 x 0.5	1.8 x 1.8, R. I.	1 x 1, R. I.	0.5 x 0.5	N. P.	
1.4 x 1.4, R. I.	N. P.	1.8 x 1.8	0.2 x 0.2, red spot	0.8 x 0.8, raised nod.	N. P	
1.7 x 1.7, R. I.	0.8 x 0.8	2 x 2, R. I.	N. P.	0.5 x 0.5	0.3 x 0.3	
1.2 x 1.2, I. R.	N. P.	1.5 x 1.5, R. I.	N. P.	N. P.	N. P	
....	....	Nodule				
1.6 x 1.6	0.8 x 0.8	1.8 x 1.8, R. I.	1.5 x 1.5, R. I.	0.5 x 0.5	N. P.	
Red area, no induration	N. P.	1.4 x 1.4, R. I.	1.1 x 1.1, R. I.	0.5 x 0.5	N. P.	
....	....	Nodule	....	Nodule small		
0.8 x 0.8, Red area, no induration	0.5 x 0.5	1.8 x 1.8, R. I.	0.6 x 0.6, red	0.5 x 0.5		
0.9 x 0.9, Red area, no induration	N. P.	1.8 x 1.2, R. I.	N. P.	0.4 x 0.4		
....	....	Pin-head nodule	....	Pin-head nodule		
1.3 x 1.3, yellow center	0.3 x 0.3	1.8 x 2.8, yellow center	0.9 x 0.9	1 x 1, R. I.	N. P.	
0.9 x 0.9	N. P.	2.1 x 2.1, R. I.	1.3 x 1.3	0.5 x 0.5, R. I.	N. P.	
....	....	1.5 x 1.5, Nodule	Nodule	0.5, nodule		

TABLE 4—*Continued*

RESULTS OF INTRACUTANEOUS TESTS WITH TYPHOIDIN (GROUP 3)

Rabbit	Immunization	Last Infection	Serologic Tests		Hours	Typhoidin IIa 1:100 (cm.)	Typhoidin Ia 1:100 (cm.)
			Agglutination	Complement-Fixation			
717	B. paratyphosus A, Fraser 5 injections	Sept. 26, 1916	1:20,000	0.001	24 48 120	1.9 x 1.9, R. I. 1.5 x 1.5, R. I. Nodule	1.5 x 1.5, R. I. 1.2 x 1.2, R. I. Nodule
718	B. typhosus Kearney Gallbladder-injection	Sept. 16, 1916	1:2000	0.01 —0.0075	24 48 120	1 x 1, R. I. 0.7 x 0.7, nodule	1.1 x 1.1, R. I. 0.7 x 0.7, R. I. ....
719	B. paratyphosus A, Vaughn	Sept. 16, 1916	1:10,000	0.0005	24 48 120	0.8 x 0.8, W. D. 1.8 x 1.8, red area	1.1 x 1.1, red 1 x 1 ....
741	B. paradysenteriae Napa Gallbladder-injection	Sept. 26, 1916	1:200 —400		24 48 120	1.5 x 1.8, red area 0.6 x 0.6, nodule	0.7 x 0.7 0.6 x 0.6, nodule raised ....
749	Control		1:100		24 48	1.3 x 1.3, red area 1.4 x 1.4, red area	1.2 x 1.2, red area 0.2 x 0.2, nodule
750	Control		1.40		24 48	1 x 1, red N. P.	1.5 x 1.5, red area N. P.
751	Control		1:20		24 48 120	1.3 x 1.3, red 0.9 x 0.9, nodule	0.8 x 0.8, R. I. 0.4 x 0.4, R. I. ....
752	Control		1:20		24 48	N. P. N. P.	N. P. N. P.
753	Control		1:20		24 48	N. P. N. P.	N. P. N. P.

The results of this test are summarized in Table 4. The cutaneous response of the rabbits to the glycerin extracts of B. typhosus and that to the pure typhoprotein in this experiment were visibly not of the same intensity, and the reactions did not run parallel in the different animals. On account of the marked effect of this latter preparation on the control animals, the results obtained with Typhoidin V in a dilution of 1: 100 must be excluded from consideration. The pure typhoprotein in dilution of 1: 1000 (0.0001 gm. = 0.1 mg.) is therefore more suitable for our comparative studies.

TABLE 4—*Continued*

RESULTS OF INTRACUTANEOUS TESTS WITH TYPHOIDIN (GROUP 3)

Typhoidin I-b 1:100	Control-Powder 1:25	Typhoidin V		Tuberculin B. C. 1:50	Pork Protein 1:10	Remarks
		1:100	1:1000			
(cm.)	(cm.)	(cm.)	(cm.)	(cm.)	(cm.)	
1.2 x 1.2, R. I. 0.8 x 0.8, R. I. Nodule	0.8 x 0.8, R. I. 0.5 x 0.5, R. I.	2.5 x 1.9, R. I. 2.4 x 2.4 Nodule	Diffuse red area 0.6 x 0.6	Diffuse reddish area N. P. Nodule	N. P. N. P.	Lost in weight
1.2 x 1.2, R. I. 0.4 x 0.4, R. I.	N. P. N. P.	1.7 x 1.7 1.1 x 1.1	1.2 x 1.2, red 1 x 1	1.1 x 1.1 0.4 x 0.4 Nodule	....	Suppurative cholecystitis Nov. 18, 1916
1 x 1, R. I. 0.6 x 0.6	0.5 x 0.5 0.4 x 0.4	2.2 x 2.2, D. O. 2.1 x 2.1 Nodule	2.5 x 2.5, edema 1.5 x 1.5	0.7 x 0.7 0.7 x 0.7 Nodule	N. P. N. P.	
1.8 x 1, R. I., edema 0.6 x 0.6, raised nod.	0.2 x 0.2 N. P.	About 4.5 x 2.8, D. O.	About 4.5 x 2.8, D. O.	0.8 x 0.8 0.4 x 0.4	N. P. N. P.	
0.7 x 0.7 0.2 x 0.2, nodule	N. P. N. P.	1.6 x 1.6, R. I. 1.9 x 1.9, R. I., nodule 2 x 2	0.4 x 0.4 N. P.	0.4 x 0.4 0.3 x 0.3	N. P N. P.	
0.8 x 0.8, red N. P.	N. P. N. P.	1.2 x 1.2 1 x 1, nodule	N. P. N. P.	0.6 x 0.6, red 0.5 x 0.5, nodule		
0.9 x 0.9, red 0.8 x 0.8, red	0.6 x 0.6 .... N. P.	3.8 x 3.8, D. O. 2.8 x 4.8, D. O.	3.8 x 3.8, D. O. 2.8 x 4.8, D. O.	0.8 x 0.8 0.4 x 0.4	N. P. N. P.	
N. P. N. P.	N. P. N. P.	2.1 x 1.6 1.7 x 1.7	0.6 x 0.6 0.2 x 0.2	0.5 x 0.5 0.5 x 0.5	N. P. N. P.	
N. P. N. P.	N. P. N. P.	1.5 x 1.5 1.4 x 1.4	0.5 x 0.5 0.5 x 0.5	0.5 x 0.5 0.3 x 0.3	N. P N. P.	

Of 3 rabbits (691, 692, and 716) immunized against B. typhosus, only 1 rabbit (716) gave a well-defined fair-sized reaction after 24 hours, which persisted for 2 or 3 days. Animals 691 and 692 gave, with typhoprotein, reactions which differed very little from those of the controls, but they reacted distinctly to one or two of the glycerin extracts. The crude unfiltered (II^a) and also the unpurified (I^a) preparations were visibly more active, but correspondingly irritant to the skin of several control rabbits (749, 750, 751). These reactions are in many respects similar to those in Experiment-group 4, soon to be discussed.

There is not much difference in action between the typhoidin preparations I^a and I^b. The purified extract I^b provoked somewhat less intensive reactions than I^a. In considering the typhoidin I^a as the best of the glycerin-extract preparations, we found that Rabbit 692 gave a marked persistent reaction, which was surpassed in intensity, however, by that of the more recently immunized rabbit, 716.

Quite in accord with our previous observations was the finding that rabbits highly immunized with bacteria other than typhoid — for example, in this experiment B. paratyphosus A (Rabbits 717 and 719) — will give strong reactions to glycerin extracts and, in a slight but definite degree (719), to typhoprotein also. The only rabbit (711) intensively immunized with paradysentery organisms reacted like the controls, both to extracts and to typhoprotein.

The skin reactions of the rabbits infected by gallbladder-inoculations are not conclusive. Rabbits 705 and 718 with a fair degree of serum immunity gave well-defined, well-indurated reactions to the filtered glycerin extract; the typhoid-infected rabbit (718) reacted positively also to typhoprotein.

The dysentery rabbits (703, 704, 706, 741) reacted slightly, if at all, to the nonpurified glycerin extracts, but gave well-defined and marked reactions to the typhoprotein and the purified typhoidin I^b. Most of the areolae were well defined and persisted in some rabbits for at least 48 hours. These rabbits possessed a slight degree of immunity because the dysentery infection remained in the biliary passages and very little bacterial protein was probably absorbed to produce the condition of generalized nonspecific hypersensitiveness to bacterial glycerin extracts so commonly observed throughout our experiments in highly immunized animals.

In our opinion, it is not a mere coincidence that in these paradysentery-infected rabbits the reactions to typhoprotein ran parallel with those to the concentrated and purified typhoidin I^b. This observation supports our views that this cutaneous hypersensitiveness is due to substances of the bacterial body and not to metabolic products developed in the broth by the growth of the microorganism. In the purified extract powders and the typhoprotein, these substances are more concentrated than in the crude and unfiltered preparations.

The bovine tuberculin B. C. produced reactions which can be compared with those produced by other colloidal suspensions. As a rule, the rabbits which reacted well to unfiltered glycerin extracts also produced areolae with tuberculin; for example, 716, 718, and 691. In the same animals the reaction persisted for many days in the form of a small aseptic tuberculoma. The high concentration of the tuberculoprotein is probably in part responsible for these reactions; they lack specificity, for some of the control rabbits reacted similarly, tho not so intensely.

Pork protein was inactive in all the rabbits tested.

With Group 3 the results were as follows: A filtered glycerin rabbit-culture extract of B. typhosus called forth distinct reactions in typhoid- and paratyphoid-immune rabbits; as a rule, the areolae were smaller than those noted for the unfiltered preparation. Filtered typhoidin powder is also less irritant to normal healthy rabbits than the unfiltered product. The purified and more concentrated typhoidin is not very irritant to normal rabbits; it gives smaller reactions and behaves somewhat as the pure typhoprotein does. This again indicates that the amount of specific substance demonstrable by complement-fixation test, or the so-called antigenic value of a typhoidin preparation, does not always indicate the suitability of the powder as an antigen for skin tests. This is particularly true for the typhoprotein with a value of 333 e. u. per milligram. The skin reactions to this highly potent preparation in dilutions inert for control animals, were small, sometimes indistinct, and therefore frequently inconclusive. On the other hand, concentrated suspensions of typhoprotein were nonspecific. On account of their high concentration in bacterial protein, they acted as severe irritants and produced extensive pyogenic reactions in immunized and normal rabbits.

It is quite apparent that pure bacterial proteins are capable of producing skin reactions in immune or infected animals, just as Deehan[13] has observed in typhoid cases in man. The results thus far available from this one test, indicate only that these reactions can have a certain degree of specificity as judged by the intensity of the reaction in typhoid- or paratyphoid-immune rabbits. In choosing another, perhaps more purified, preparation, it may be possible to obtain even more specific reactions which would be conclusive evidence that the cutaneous hypersensitiveness is due to sensitization with some simple or complex element of the typhoprotein. From a diagnostic viewpoint it seems paradoxical, however, that in the rabbit the pure bacterial protein of the typhoid bacillus should be less suitable for cutaneous tests than the purified glycerin extract. Previous observations have shown that suspensions of tubercle bacilli are better antigens than tuberculin for diagnostic skin tests in tubercular rabbits. Additional experiments, now in progress, will throw more light on this interesting finding.

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[13] Univ. of Penn. Med. Bull., 1909, 22, p. 192.

From the animals of Experiment-group 3 no further information can be drawn concerning the nature of the nonspecific reactions in highly immunized rabbits; it is however quite apparent that the pure typhoprotein which does not contain substances of the broth, gives reactions with dysentery-infected or with immune rabbits. These reactions are, as already described, paralleled by those to the purified typhoidin I^b. The latter preparation proved to be nontoxic in some of the rabbit-inoculation experiments, 0.2 gm. inoculated intravenously producing slight loss in weight and yet giving rise to considerable amounts of agglutinins (see Chart 1, 1st paper). The reactions cannot, therefore, be ascribed to the action of the endotoxin. They suggest, however, a condition of cutaneous hypersensitiveness, which is probably a form of sensitization to certain elements of the bacterial proteins common to most microorganisms.

The evidence collected in Group 3 did not appear sufficient to justify the conclusion that the cutaneous typhoidin reaction is due to sensitization, and we attempted, therefore, the passive transfer of the hypersensitiveness.

Gay and Claypole[4] reported experiments in which they succeeded in transferring the susceptibility to typhoidin from an immune to a normal animal. They proved that the skin reaction is the result of an interaction of antigen and antibodies. We repeated these experiments successfully in 2 exactly similar experiments by inoculating 20 c.c. of rabbit typhoid-immune serum into the peritoneal cavity of a normal rabbit and testing the latter with typhoidin and similar preparations 24 hours later.

The cutaneous hypersensitiveness was present, not only to the typhoid, but also to the fowl-typhoid antigen. The reactions to extracts of B. dysenteriae were inconclusive. Further experiments along the same lines are in progress with the main object of finding out whether specific hypersensitiveness alone, or together with the nonspecific factors, is transferable from immune, hyperimmune, or infected rabbits to normal animals of the same species, and also to guinea-pigs.

EXPERIMENT-GROUP 4

In our first communication we called attention to the accidental observation that normal healthy rabbits which had served as controls in our typhoidin tests would give positive skin reactions when retested, within 1 to 2 months, on another portion of the skin. This acquired cutaneous hypersensitiveness was present for at least 2 months in some rabbits. We suspect that the retests were in part responsible for renewing the hypersusceptibility to typhoidin. Group 4 illustrates some of the reactions recorded.

Four rabbits (607, 608, 609, and 640) which had served as controls in Group 1, Series 1 and 2, and in Group 1, 3rd paper, were tested along with a number of immune and normal rabbits which were being used in connection with some other experiments. These rabbits gave, on the first test, very slight or negative reactions. In neither immune nor normal animal did the diameter of the areola exceed 0.5 cm. in the first 24 hours. Only in Rabbit 609 had the agglutination titer changed between the 1st and 2nd tests. Specific complement-fixation antibodies could not be demonstrated in either rabbit. The typhoidin preparations P.C. and E. used in the group, were suitable antigens and have been fully considered in the experiments in which these rabbits were included.

In Tables 5 and 6 the results are summarized. Rabbits 607 and 608 reacted to the intracutaneous typhoidin test in the same manner as immunized or recovered animals. The reactions were well marked and correspondingly persistent for 48 hours. It is rather interesting to note that Rabbit 608 — which received quantitatively more bacterial extracts at the 1st test on February 23 — showed not only better reactions with typhoidin than did Rabbit 607, but also noteworthy group reactions with paratyphoidin-A and -B, and B.-coli extracts. The few milligrams of antigen were apparently sufficient to produce general cutaneous hypersensitiveness.

In a similar manner, Rabbits 609 and 640 reacted to Typhoidin E, and the other extracts used in Group 1, Series 2. The results are not so striking, on account of the different character of the typhoidin preparations used in this experiment.

In comparison with the controls 662 and 663 (Table 2), the sensitized control rabbits 609 and 640 gave good reactions to broth typhoidin. In every respect the areolae appeared to be identical in character with those on the typhoid-immune rabbits tested simultaneously.

The experience summarized in Group 4 furnishes the proof that cutaneous hypersensitiveness to typhoidin is readily acquired by rabbits.

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[14] Compt. rend. Soc. de biol., 1907, 63, p. 296.

TABLE 5

RESULTS OF INTRACUTANEOUS TESTS WITH TYPHOIDIN (GROUP 4)
DATE OF TESTS: APRIL 10, 1916

Rabbit	Previous Test	Serologic Tests, April 10, 1916		Hours
		Agglutination	Complement-Fixation	
607	Control Feb. 23, 1916 Typhoidin Kuhn Fowl Typhoidin, A. P. Smith Staphylococcin	1:60	0.05 (50%)	16 24 48 72
608	Control Feb. 23, 1916 Typhoidin, Rawlings Dysentery Do. B. coli	1:20	0.1 (50%)	16 24 48 72

Calmette, Breton, and Petit[14] reported that nontubercular rabbits would give positive ophthalmic reactions after the subcutaneous injection of tuberculin; these authors explain this observation as a phenomenon of local sensitization. Similar conditions apparently develop as a result of intravenous or subcutaneous injections of typhoidin. In 3 experiments we could show that in this manner the application of typhoidin preparations in the amounts of 0.01 gm. produced cutaneous hypersensitiveness to the same or to similar bacterial extracts. One of these experiments is illustrated in Table 7.

The experimental data thus far collected, strongly support the conception that the typhoidin reaction is in all probability the result of a process of sensitization. One must reserve for further investigations the determination of the elements of the typhoid bacillus which are responsible for this sensitization, and great difficulty is to be

TABLE 6

RESULTS OF INTRACUTANEOUS TESTS WITH TYPHOIDIN (GROUP 4)
DATE OF TESTS: APRIL 24, 1916

Rabbit	Previous Test	Serologic Tests, April 24, 1916		Hours
		Agglutination	Complement-Fixation	
609	Control Feb. 23, 1916 Typhoidin 3 Staphylococcin 4 Paratyphoidin	1:40-1:100	0.05 (50%)	24 48
640	Control April 10, 1916 Fowl typhoidin Staphylococcin Paratyphoidin B Dysentery Do.	1:40-1:100 (Fowl typhoidin) (1:1000)	0	24 48

TABLE 5—*Continued*

RESULTS OF INTRACUTANEOUS TESTS WITH TYPHOIDIN (GROUP 4)
DATE OF TESTS: APRIL 10, 1916

Typhoidin P. C.		B. Paratyphoid A 1:100	B. Coli	Paratyphoidin B 1:100	Control Powder
1:50	1:100				
(cm.)	(cm.)	(cm.)	(cm.)	(cm.)	(cm.)
1.6 x 1.6	Red area	Red area	Diff. red area	Red spot	1.4 x 1.4
1.9 x 1.9	Red spot	1.6 x 1.6	Diff. red area	Red spot	Red area
2.2 x 2.2	N. P.	Red area	0.2 x 0.2	N. P.	N. P.
N. P.	N. P.	Red spot		N. P.	N. P.
1.4 x 1.4	1 x 1	1.5 x 1.5	1 x 1	Red area	N. P.
2 x 2	1.6 x 1.6	1.4 x 1.4	0.9 x 0.9	Diff. red area	Red spot
1.8 x 1.8	1 x 1	1 x 1	1 x 1	1.7 x 1.7	Red spot

expected as long as we know so little about the nature of bacterial anaphylaxis in general.

From a diagnostic viewpoint it is important to remember that cutaneous hypersensitiveness is readily acquired by rabbits. This fact deprived us of the means by which we originally intended to follow the gradual disappearance of the hypersusceptibility in its relation to the resistance in an infection. Thus far it appears that the rabbit is unsuitable for the study of cutaneous sensitization.

DISCUSSION

The variability and suitability of the various glycerin extracts of typhoid and other bacterial cultures have been discussed in connection with each experiment group, and it has been pointed out that, as a rule, rabbits immunized against B. typhosus will give well-defined positive

TABLE 6—*Continued*

RESULTS OF INTRACUTANEOUS TESTS WITH TYPHOIDIN (GROUP 4)
DATE OF TESTS: APRIL 24, 1916

Typhoidin E		Control Powder	Staphylococcin	Paratyphoidin A 1:100	Paratyphoidin B 1:100
1:100	Undiluted Broth				
(cm.)	(cm.)	(cm.)	(cm.)	(cm.)	(cm.)
1 x 1	1.2 x 1.2	N. P.	N. P.	N. P.	N. P.
0.9 x 0.9	1 x 1	N. P.	N. P.	N. P.	N. P.
1.6 x 1.6	1.6 x 1.6	0.5 x 0.5	Red spot	N. P.	1.2 x 1.9
1.4 x 1.4	1.8 x1.8	Red spot	0.5 x 0.5	0.8 x 0.8	1:4 x 1.4

TABLE 7

RESULTS OF INTRACUTANEOUS TESTS WITH TYPHOPROTEIN

Rabbit	Injection	Agglutinins	Hours	Typhoprotein 323	Typhoprotein Nelson	B.-Coli Protein	Army Vaccine	Staphylococcus Protein
742	Typhoidin Ia 40 days previous to test	1:200	24	1.5 x 1.4, R. I.	0.8 x 0.8	1.3 x 1.3	0.6 x 0.6	0.6 x 0.6
			48	1.1 x 1.1, R. I.	N. P.	0.5 x 0.5	0.2 x 0.2	0.4 x 0.4
743	Typhoidin IIa (Nelson) 40 days	1:40	24	1.2 x 1.2, red area	1.3 x 1.3, R. I.	1.2 x 1.2, R. I.	1.9 x 1.9, red only	2.5 x 2.5, red
			48	N. P.	0.2 x 0.2	N. P.	0.5 x 0.5	1.2 x 1.2, red
744	Typhoidin Ic 40 days	1:100	24	1.8 x 1.7, R. I.	0.5 x 0.5, R. I.	1.1 x 1.1, R. I.	1.5 x 1.5, R. I.	0.8 x 0.8, R. I.
			48	1.3 x 1.3	0.6 x 0.6, R. I.	0.8 x 0.9, R. I.	0.5 x 0.5, R. I.	1.0 x 1.0, R. I.
757	Control		24	0.5 x 0.5, red spot	N. P.	N. P.	0.3 x 0.3	1.2 x 1.2, red
			48	0.4 x 0.4, red area	N. P.	N. P.	0.4 x 0.4, nodule	1 x 1, red
758	Control		24	N. P.	N. P.	N. P.	N. P.	N. P.
			48	N. P.	N. P.	N. P.	N. P.	N. P.

skin-reactions which may be considered specific. These findings therefore confirm those made by Gay and his co-workers[4] on rabbits.

Observations made on rabbits immunized with bacteria other than typhoid, however, apparently show that the skin can react to various bacterial extracts. It is at once clear from the repeated occurrence of these reactions that they are nonspecific and cannot well be used as indices of immunity, for which this biologic test was primarily intended.

An explanation is best attempted on the basis that the typhoidin and similar reactions in rabbits are anaphylactic in nature and the result of an interaction of antigen and antibody. The observations of Gay and his co-workers,[4] and our own experience, furnish evidence that such a conception is fully in harmony with the experimental evidence thus far collected. Typhoidin prepared with typhoid autolysates gives, in proper doses, skin reactions on immune or infected rabbits, which have every appearance of being specific. The complement-fixation tests with glycerin-extract preparations of the typhoid bacillus gave information with reference to the antigenic value of this typhoidin, and it was definitely established that protein substances, which gave complement-fixation only with a specific antiserum, were always present. Naturally, in these tests the typhoidin gave also group reactions with the serum of rabbits which had been immunized with closely allied organisms. As a rule, the extracts were suitable specific antigens, even if they did not show as high an antigenic value as the pure typhoprotein. Probably dilution of the antigenic substances by the inactive precipitable polypeptids and other broth elements is in part responsible for this condition.

The suitable preparations are inactive on the skin of normal healthy rabbits, or they produce areolae which are not indurated and less than 1 cm. in diameter. On the other hand, in very small amounts, they produce intense reactions in immunized or infected rabbits. The intensity of the reaction runs parallel, in most of the tests, to the degree of immunization; the greater the concentration and number of injections of the bacterial suspension which has been inoculated, the stronger the reaction. The hypersensitiveness can be transferred to normal rabbits. Repeated intracutaneous tests produce a local cutaneous hypersensitiveness to the bacterial substances which are present in the extracts. In this connection it is of interest that such cutaneous hypersensitiveness can be present without appreciable increase or change of the serum antibodies.

Recent studies reported by Kolmer and his associates[6] support these observations perfectly. He found in various infectious diseases of man and animal that cutaneous hypersensitiveness may exist without demonstrable antibodies in the body fluids.

The logical assumption from all of these facts is that cutaneous hypersensitiveness is the result of bacterial-protein sensitization. In the light of the conception of specificity it is difficult, however, to understand the many typhoidin reactions in rabbits treated with microorganisms biologically and immunologically different from the typhoid bacillus. The nearest explanation would, in some instances, at least, be the primary toxic action of the glycerin extractives on the delicate rabbit skin. We feel that our preparations have been carefully tested on normal rabbits and usually found inert in those dilutions which caused reactions in the immune rabbits.

There is the possibility — analogous to our findings in normal rabbits — that some of these rabbits possessed a marked individual hypersusceptibility to toxic albumoses of the extractives. It is, however, also evident from the tables that the immunized rabbits, which reacted apparently nonspecifically to bacterial proteins, showed no skin reaction with concentrated peptone solutions, which were always used in the control powders.

The fact that typhoid-immune rabbits react with paratyphoid, dysentery, and sometimes with other vegetable-protein extracts, and that rabbits immune to fowl typhoid, B. bipolaris-septicus, or those infected with tuberculosis, respond to typhoidin and similar products, suggests that the hypersensitiveness is the result of changes produced in the body of the animal by the process of immunization or infection.

In connection with the infections, additional information has recently come from the confirmatory tests of Dr. Traum, of the University of California, who was kind enough to carry out this observation on tubercular cattle and guinea-pigs. A nontoxic typhoidin in 10% solution produced, on intradermal injection into tubercular cattle, reactions which were similar in intensity to those obtained simultaneously with tuberculin. In the experience of Dr. Traum and ourselves, tubercular guinea-pigs also give reactions, which differ, however, from the true tuberculin reaction in size and induration.

The literature contains many similar observations that intercurrent diseases and local or general infections frequently cause cutaneous or general hypersensitiveness to various bacterial proteins. Mareck,[15] for instance, found that horses suffering from pneumonia or scabies, would give positive ophthalmic or thermic reactions with typhin, a preparation similar to, if not identical with, our typhoidin. Südmersen[16] observed typical local and general tuberculin and mallein reactions in horses which were free from these infections, but which had been inoculated with cultures of various bacteria for the preparation of antisera. Furthermore, Römer[17] was able to kill tubercular guinea-pigs with a protein substance of the pneumobacillus of Friedlander and B. pyocyaneus which produced only a temperature reaction in healthy guinea-pigs. Petruschky[18] observed in tubercular persons general hypersensitiveness to various bacterial products, and he even goes so far as to state that the hypersensitiveness to tuberculosis is due to a specific, and a nonspecific component. And again, luetin reactions have been produced in late syphilis by Boas and Ditlevsen[19] with gonococcal and colon-bacillus suspensions. Several years ago one of us[20] reported, in confirmation of the observations of Bang[21] and

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[15] Deutsch. tierärztl. Wchnschr., 1916, 24, p. 33.
[16] Quoted by Mareck (see Reference 15).
[17] Wien. klin. Wchnschr., 1891, 4, p. 835.
[18] Ergebn. d. Inn. Med. u. Kinderheilk., 1912, 9, p. 557.
[19] Arch. f. Dermat. u. Syph., 1913, 66, p. 852.
[20] Meyer, Jour. Med. Research, 1913, 29, p. 180.
[21] Centralbl. f. Bakteriol., I, O., 1908, 51, p. 450.

Horné,[22] that paratubercular cattle react locally and generally to an inoculation of avian tuberculin. This response certainly cannot be regarded as a group reaction of the avian tuberculoprotein with the anti-paratuberculoprotein, because the two organisms are only biologically related, as the tubercle bacillus is to the leprosy organism. Finally, Paisseau and Fixier[23] observed positive tuberculin reactions in typhoid patients, on the intracutaneous injection of this antigen, this reaction becoming negative, however, during convalescence.

Through the recent studies of Wells and Osborne[24] it is known that the specificity of an anaphylactic protein reaction is dependent on the chemical constitution of the reacting substances. One and the same protein molecules can, according to their conception, contain two or more specific substances which are capable of sensitizing the animal body. We were therefore not entirely unsupported when in previous paragraphs we expressed the opinion that the skin reactions noted with typhoidin in rabbits immunized against B. bipolaris, are due to sensitization with protein groups of identical chemical composition which, in all probability, are common to most of the bacteria—or even, perhaps, to the vegetable proteins in general. In the light of the observations of Wells and Osborne, it may be correct to assume that the complex typhoprotein contains parts of protein groups which are common also for the yeast or the B.-bipolaris-septicus protein. Extensive immunization or infection will finally give rise, aside from cutaneous hypersensitiveness, to the more prevalent specific typhoid proteins as well as to local or general hypersusceptibility to other protein fractions or complexes.

Furthermore, it has been shown by Wells and Osborne[25] that the antisera of rabbits which had received small doses of hordein gave complement-fixation only with this substance, but when larger doses were introduced, the reaction occurred also with gliadin, malt-proteoses, and so on. Such observations have also been made by us, and others, in connection with interagglutination tests in the paratyphoid group of bacteria.

It is possible that the same principle can be applied to the phenomena of anaphylaxis or sensitization in general; namely, that the degree of protein immunity has considerable influence on the specificity. An animal with a low immunity will only give reactions with the homologous, chemically (or biologically) analogous antigen; a highly immune or infected animal will also react with heterologous antigens which contain chemically closely related protein elements.

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[22] Berl. tierärztl. Wchnschr., 1908, 23, p. 235.

[23] Compt. rend. Soc. de biol., 1909, 66, p. 877.

[24] Jour. Infect. Dis., 1913, 12, p. 341.

[25] Ibid., 1914, 14, 375.

The nonspecific skin reactions observed in our experiments may be explained on this basis of sensitization. They are the result of a prolonged and intensive immunization with bacterial proteins which, considered as complexes, probably contain groups of protein elements found in all bacteria and perhaps in vegetables in general. The same mechanism may be at work in active disease and in infection. The specific cutaneous hypersensitiveness to homologous antigens, which is, as a rule, a fine indicator of general sensitiveness, apparently becomes, in a certain percentage of rabbits, gradually veiled during the course of immunization or disease by the development of nonspecific sensitization to heterologous antigens. The most intense nonspecific skin reactions were seen in animals which had been immunized with living organisms or had received more than 10 large intravenous injections.

It is necessary to mention here, however, that this form of sensitization does not occur with absolute regularity in every hyperimmune animal. And again, some observations indicate that over-immunization tends to reduce or wipe out cutaneous hypersensitiveness, just as it affects the serum antibodies. (This is particularly true for immunization with organisms of the B.-dysenteriae group.) How far individual factors and parasitic infections in rabbits influence the chances of nonspecific cutaneous hypersensitiveness, must still be investigated.

The explanation by Stokes[10] of nonspecific skin reaction deserves further careful investigation on animals. We called attention to some atypical reactions the explanation of which by the assumption of a mechanism similar to that advanced by this writer, seems plausible. We failed to note in the rabbit the characteristic cycle of response on inoculation of colloids like agar, and therefore we lack experimental evidence to support the conception of Stokes.

The nonspecific reactions do not impair the diagnostic value of skin tests as a means of detecting hypersensitiveness to bacterial proteins, because as a rule the areola of the reaction to the specific antigen is larger and more intense than those of the reactions to the heterologous products. But they materially influence the conception that cutaneous hypersensitiveness can be used as an index of defensive activity of the part of the body fluids, or cells, against the bacterial protein or against the living organism with which the skin reaction was obtained. How far our observations and explanations have a bearing on the diagnostic value of these tests in man, we are not at present prepared to say. Sufficient observations are published in the literature to show that, at least in tubercular persons, a nonspecific reaction with typhoidin may be expected in a certain percentage of cases.

CONCLUSIONS

Cutaneous hypersensitiveness of rabbits to typhoidin and similar preparations is, in all probability, the result of sensitization with typho- or similar bacterial proteins. The hypersusceptibility to extract preparations of cultures can be passively transferred, and small amounts of typhoidin are capable of producing local sensitization of the skin to subsequent injections of the same, or similar, antigens.

In a fairly high percentage of hyperimmune or infected rabbits, nonspecific skin reactions follow the injection of extracts of pure bacterial proteins or microorganisms neither biochemically nor immunologically related to the organisms with which the animal has been treated. Some typhoid-immune rabbits show nonspecific reactions with paratyphoid and B.-coli extracts, which can be considered as group reactions; but frequently such animals, when highly immunized, will also give reactions with heterologous antigens not in the least related to B. typhosus. These reactions may be explained as the probable result of nonspecific sensitization with protein substances common to all bacteria or vegetables in general.